

09/546,857 Listing of Claims:

Claims 1-56 (canceled)

⁴⁴
Claim ~~57~~ (currently amended): A variant of human VEGF comprising amino acid substitutions D63S, G65M, and L66R, wherein the variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

⁴⁵
Claim ~~58~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~57~~.

⁴⁶
Claim ~~59~~ (previously presented): A vector comprising the nucleic acid of claim ~~58~~.

¹
Claim ~~60~~ (currently amended): A variant of human VEGF comprising one or more non-conservative amino acid substitution(s) at residues 63 to 66 and one or more amino acid substitution(s) at residues 18, 21, 22, or 25, wherein the VEGF variant exhibits selective binding affinity for KDR-receptor a higher KDR to Flt-1 binding ratio as compared with native VEGF.

¹⁸
Claim ~~61~~ (currently amended): The VEGF variant of claim ~~60~~, wherein at least one of the amino acid substitution(s) comprises D63S, G65M, or L66R.

²
Claim ~~62~~ (currently amended): The VEGF variant of claim ~~60~~, wherein at least one of the amino acid substitution(s) comprises M18E, Y21L, Q22R, or Y25S.

³
Claim ~~63~~ (previously presented): The VEGF variant of claim ~~60~~, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

²⁷
Claim ~~64~~ (previously presented): The VEGF variant of claim ~~60~~ wherein the amino acid substitutions comprise D63S, G65M, and L66R.

Claim ²⁸~~65~~ (currently amended): The VEGF variant of claim ²⁷~~64~~, wherein at least one of the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim ¹⁹~~66~~ (currently amended): The VEGF variant of claim ¹⁸~~64~~, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim ²⁹~~67~~ (currently amended): ~~A~~The VEGF variant of claim ²⁸~~66~~, comprising ~~one of the~~ following combinations of amino acid substitutions:

- ~~(a) M18E, D63S, G65M, and L66R;~~
- ~~(b) Y21L, D63S, G65M, and L66R;~~
- ~~(c) Q22R, D63S, G65M, and L66R;~~
- ~~(d) Y25S, D63S, G65M, and L66R;~~
- ~~(e) M18E, Y21L, D63S, G65M, and L66R;~~
- ~~(f) M18E, Q22R, D63S, G65M, and L66R;~~
- ~~(g) M18E, Y25S, D63S, G65M, and L66R;~~
- ~~(h) Y21L, Q22R, D63S, G65M, and L66R;~~
- ~~(i) Y21L, Y25S, D63S, G65M, and L66R;~~
- ~~(j) Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(k) M18E, Y21L, Q22R, D63S, G65M, and L66R;~~
- ~~(l) M18E, Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(n) M18E, Y21L, Q22R, Y25S, and D63S;~~
- ~~(o) M18E, Y21L, Q22R, Y25S, and G65M;~~
- ~~(p) M18E, Y21L, Q22R, Y25S, and L66R;~~
- ~~(q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;~~
- ~~(r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;~~
- ~~(s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or~~
- ~~(t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.~~

Claim ⁵~~68~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~60~~.

⁴
Claim ~~69~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~68~~³.

³¹
Claim ~~70~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~69~~²⁸.

³⁰
Claim ~~71~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~70~~²⁹.

⁶
Claim ~~72~~ (previously presented): A vector comprising the nucleic acid of claim ~~68~~⁵.

⁷
Claim ~~73~~ (previously presented): A host cell comprising the vector of claim ~~72~~⁶.

⁸
Claim ~~74~~ (previously presented): A composition comprising the VEGF variant of claim ~~68~~ and a carrier.

⁹
Claim ~~75~~ (previously presented): The composition of claim ~~74~~⁸, wherein the carrier is a pharmaceutically acceptable carrier.

¹⁰
Claim ~~76~~ (previously presented): An assay for detecting KDR receptor, comprising contacting an isolated cell or tissue with a VEGF variant of claim ~~68~~ and assaying for binding of the VEGF variant to the cell or tissue.

¹¹
Claim ~~77~~ (previously presented): A method for stimulating phosphorylation of a KDR receptor, comprising contacting a cell with a VEGF variant of claim ~~68~~ in amount effective to stimulate phosphorylation of the KDR receptor.

¹²
Claim ~~78~~ (previously presented): A method for stimulating MAP kinase activation, comprising contacting a cell with a VEGF variant of claim ~~68~~ in amount effective to stimulate phosphorylation of MAP kinase.

¹³
Claim ~~78~~ (previously presented): A method for stimulating PLC-gamma activation, comprising contacting a cell with a VEGF variant of claim ~~60~~ in amount effective to stimulate phosphorylation of PLC-gamma.

¹⁴
Claim ~~80~~ (previously presented) A method for stimulating PI 3'-kinase activation, comprising contacting a cell with a VEGF variant of claim ~~60~~ in amount effective to stimulate phosphorylation of PI 3'-kinase.

¹⁵
Claim ~~81~~ (previously presented): A method for stimulating vasculogenesis or angiogenesis, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim ~~60~~.

¹⁶
Claim ~~82~~ (previously presented): A method for promoting the migration of endothelial cells, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim ~~60~~.

⁴⁷
Claim ~~83~~ (currently amended): A variant of human VEGF comprising two or more amino acid substitutions at residues 17 to 25, wherein the VEGF variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

⁴⁸
Claim ~~84~~ (previously presented): The VEGF variant of claim ~~83~~, wherein the amino acid substitutions comprise two or more amino acid substitutions at residues 18, 21, 22, or 25.

⁴⁹
Claim ~~85~~ (currently amended): The VEGF variant of claim ~~83~~, wherein at least one of the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

⁵⁰
Claim ~~86~~ (previously presented): The VEGF variant of claim ~~83~~, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

⁵²
Claim ~~87~~ (previously presented): The VEGF variant of claim ~~83~~⁴⁷, wherein the amino acid substitutions comprise F17L, M18E, Y21F, Q22K, and Y25S.

⁵³
Claim ~~88~~ (previously presented): The VEGF variant of claim ~~83~~⁴⁷, wherein the amino acid substitutions comprise F17L, M18E, Y21F, Q22E, and Y25I.

⁵⁴
Claim ~~89~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~83~~⁴⁷.

⁵¹
Claim ~~90~~ (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim ~~83~~⁵⁰.

¹⁷
Claim ~~91~~ (previously presented): The VEGF variant of claim ~~60~~¹, wherein the amino acid substitutions further comprise a substitution at residue ~~17~~¹⁷.

⁵⁵
Claim ~~92~~ (currently amended): A variant of human VEGF, comprising:

- (a) one or more amino acid substitution(s) at residues 17-25, and
- (b) one or more amino acid substitution(s) at residues 63-66;

wherein amino acid residue 60 is cysteine, and the variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

³²
Claim ~~93~~ (new): The VEGF variant of claim ~~63~~²⁸, comprising amino acid substitutions Y21L, D63S, G65M, and L66R.

³³
Claim ~~94~~ (new): The VEGF variant of claim ~~63~~²⁸, comprising amino acid substitutions Q22R, D63S, G65M, and L66R.

³⁴
Claim ~~95~~ (new): The VEGF variant of claim ~~63~~²⁸, comprising amino acid substitutions Y25S, D63S, G65M, and L66R.

³⁵
Claim ~~96~~ (new): The VEGF variant of claim ~~63~~²⁸, comprising amino acid substitutions M18E, Y21L, D63S, G65M, and L66R.

Claim ³⁶~~97~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions M18E, Q22R, D63S, G65M, and L66R.

Claim ³⁷~~98~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions M18E, Y25S, D63S, G65M, and L66R.

Claim ³⁸~~99~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions Y21L, Q22R, D63S, G65M, and L66R.

Claim ³⁹~~100~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions Y21L, Y25S, D63S, G65M, and L66R.

Claim ⁴⁰~~101~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions Q22R, Y25S, D63S, G65M, and L66R.

Claim ⁴¹~~102~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions M18E, Y21L, Q22R, D63S, G65M, and L66R.

Claim ⁴²~~103~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions M18E, Q22R, Y25S, D63S, G65M, and L66R.

Claim ⁴³~~104~~ (new): The VEGF variant of claim ²⁸~~65~~, comprising amino acid substitutions Y21L, Q22R, Y25S, D63S, G65M, and L66R.

Claim ²⁰~~105~~ (new): The VEGF variant of claim ¹⁹~~66~~, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and D63S.

Claim ²¹~~106~~ (new): The VEGF variant of claim ¹⁹~~66~~, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and G65M.

Claim ²²~~107~~ (new): The VEGF variant of claim ¹⁹~~66~~, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and L66R.

Claim ²³~~108~~ (new): The VEGF variant of claim ¹⁹~~66~~, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, D63S, and G65M.

09/546,857 Listing of Claims:

Claims 1-56 (canceled)

Claim 57 (currently amended): A variant of human VEGF comprising amino acid substitutions D63S, G65M, and L66R, wherein the variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

Claim 58 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 57.

Claim 59 (previously presented): A vector comprising the nucleic acid of claim 58.

Claim 60 (currently amended): A variant of human VEGF comprising one or more non-conservative amino acid substitution(s) at residues 63 to 66 and one or more amino acid substitution(s) at residues 18, 21, 22, or 25, wherein the VEGF variant exhibits ~~selective binding affinity for KDR receptor~~ a higher KDR to Flt-1 binding ratio as compared with native VEGF.

Claim 61 (currently amended): The VEGF variant of claim 60, wherein at least one of the amino acid substitution(s) comprises D63S, G65M, or L66R.

Claim 62 (currently amended): The VEGF variant of claim 60, wherein at least one of the amino acid substitution(s) comprises M18E, Y21L, Q22R, or Y25S.

Claim 63 (previously presented): The VEGF variant of claim 60, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 64 (previously presented): The VEGF variant of claim 60 wherein the amino acid substitutions comprise D63S, G65M, and L66R.

Claim 65 (currently amended): The VEGF variant of claim 64, wherein at least one of the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 66 (currently amended): The VEGF variant of claim ~~64~~61, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 67 (currently amended): ~~A~~The VEGF variant of claim ~~60~~65, comprising ~~one of the following combinations of~~ amino acid substitutions:

- ~~(a) M18E, D63S, G65M, and L66R;~~
- ~~(b) Y21L, D63S, G65M, and L66R;~~
- ~~(c) Q22R, D63S, G65M, and L66R;~~
- ~~(d) Y25S, D63S, G65M, and L66R;~~
- ~~(e) M18E, Y21L, D63S, G65M, and L66R;~~
- ~~(f) M18E, Q22R, D63S, G65M, and L66R;~~
- ~~(g) M18E, Y25S, D63S, G65M, and L66R;~~
- ~~(h) Y21L, Q22R, D63S, G65M, and L66R;~~
- ~~(i) Y21L, Y25S, D63S, G65M, and L66R;~~
- ~~(j) Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(k) M18E, Y21L, Q22R, D63S, G65M, and L66R;~~
- ~~(l) M18E, Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;~~
- ~~(n) M18E, Y21L, Q22R, Y25S, and D63S;~~
- ~~(o) M18E, Y21L, Q22R, Y25S, and G65M;~~
- ~~(p) M18E, Y21L, Q22R, Y25S, and L66R;~~
- ~~(q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;~~
- ~~(r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;~~
- ~~(s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or~~
- ~~(t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.~~

Claim 68 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 60.

Claim 69 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 63.

Claim 70 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 65.

Claim 71 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 67.

Claim 72 (previously presented): A vector comprising the nucleic acid of claim 68.

Claim 73 (previously presented): A host cell comprising the vector of claim 72.

Claim 74 (previously presented): A composition comprising the VEGF variant of claim 60 and a carrier.

Claim 75 (previously presented): The composition of claim 74, wherein the carrier is a pharmaceutically acceptable carrier.

Claim 76 (previously presented): An assay for detecting KDR receptor, comprising contacting an isolated cell or tissue with a VEGF variant of claim 60 and assaying for binding of the VEGF variant to the cell or tissue.

Claim 77 (previously presented): A method for stimulating phosphorylation of a KDR receptor, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of the KDR receptor.

Claim 78 (previously presented): A method for stimulating MAP kinase activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of MAP kinase.

Claim 79 (previously presented): A method for stimulating PLC-gamma activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of PLC-gamma.

Claim 80 (previously presented) A method for stimulating PI 3'-kinase activation, comprising contacting a cell with a VEGF variant of claim 60 in amount effective to stimulate phosphorylation of PI 3'-kinase.

Claim 81 (previously presented): A method for stimulating vasculogenesis or angiogenesis, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 60.

Claim 82 (previously presented): A method for promoting the migration of endothelial cells, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 60.

Claim 83 (currently amended): A variant of human VEGF comprising two or more amino acid substitutions at residues 17 to 25, wherein the VEGF variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

Claim 84 (previously presented): The VEGF variant of claim 83, wherein the amino acid substitutions comprise two or more amino acid substitutions at residues 18, 21, 22, or 25.

Claim 85 (currently amended): The VEGF variant of claim 83, wherein at least one of the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 86 (previously presented): The VEGF variant of claim 83, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 87 (previously presented): The VEGF variant of claim 83, wherein the amino acid substitutions comprise F17L, M18E, Y21F, Q22K, and Y25S.

Claim 88 (previously presented): The VEGF variant of claim 83, wherein the amino acid substitutions comprise F17L, M18E, Y21F, Q22E, and Y25L.

Claim 89 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 83.

Claim 90 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 86.

Claim 91 (previously presented): The VEGF variant of claim 60, wherein the amino acid substitutions further comprise a substitution at residue 17.

Claim 92 (currently amended): A variant of human VEGF, comprising:

- (a) one or more amino acid substitution(s) at residues 17-25, and
- (b) one or more amino acid substitution(s) at residues 63-66;

wherein amino acid residue 60 is cysteine, and the variant exhibits a higher KDR to Flt-1 binding ratio as compared with native VEGF.

Claim 93 (new): The VEGF variant of claim 65, comprising amino acid substitutions Y21L, D63S, G65M, and L66R.

Claim 94 (new): The VEGF variant of claim 65, comprising amino acid substitutions Q22R, D63S, G65M, and L66R.

Claim 95 (new): The VEGF variant of claim 65, comprising amino acid substitutions Y25S, D63S, G65M, and L66R.

Claim 96 (new): The VEGF variant of claim 65, comprising amino acid substitutions M18E, Y21L, D63S, G65M, and L66R.

Claim 97 (new): The VEGF variant of claim 65, comprising amino acid substitutions M18E, Q22R, D63S, G65M, and L66R.

Claim 98 (new): The VEGF variant of claim 65, comprising amino acid substitutions M18E, Y25S, D63S, G65M, and L66R.

Claim 99 (new): The VEGF variant of claim 65, comprising amino acid substitutions Y21L, Q22R, D63S, G65M, and L66R.

Claim 100 (new): The VEGF variant of claim 65, comprising amino acid substitutions Y21L, Y25S, D63S, G65M, and L66R.

Claim 101 (new): The VEGF variant of claim 65, comprising amino acid substitutions Q22R, Y25S, D63S, G65M, and L66R.

Claim 102 (new): The VEGF variant of claim 65, comprising amino acid substitutions M18E, Y21L, Q22R, D63S, G65M, and L66R.

Claim 103 (new): The VEGF variant of claim 65, comprising amino acid substitutions M18E, Q22R, Y25S, D63S, G65M, and L66R.

Claim 104 (new): The VEGF variant of claim 65, comprising amino acid substitutions Y21L, Q22R, Y25S, D63S, G65M, and L66R.

Claim 105 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and D63S.

Claim 106 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and G65M.

Claim 107 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, and L66R.

Claim 108 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, D63S, and G65M.

Claim 109 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, D63S, and L66R.

Claim 110 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, G65M, and L66R.

Claim 111 (new): The VEGF variant of claim 66, comprising amino acid substitutions M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

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03-23-05

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Fax Transmission | March 23, 2005

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